

1/20/98

1/20 STNFR ELISA - Mouse Serum of C3H mice injected with
 * L929 clone 39 + Primary tumor cell lines
 of C39.

+ Standard STNFR protocol
 + samples diluted in 0.1% BSA PBS-Kween
 + pNPP ~20min

	1	2	3	4	5	6	7	8	9	10	11	12
A	CLK unrad #1 1:5	CLK unrad #2 1:5	CLK unrad #3 1:5	CL39 unrad #1 1:5	CL39 unrad #2 1:5	CL39 unrad #3 1:5	CL39 3.0 Gy #1 1:5	CL39 3.0 Gy #2 1:5	CL39 3.0 Gy #3 1:5	CL39 3.0 Gy #4 1:5	TNFBPI 40ng/ml	
B												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.176	0.180	0.187	0.144	0.186	0.135	0.149	0.184	0.170	0.178	0.166	0.157
B	0.179	0.186	0.180	0.157	0.146	0.107	0.106	0.140	0.140	0.138	0.168	0.156
C	0.186	0.187	0.147	0.164	0.200	0.147	0.136	0.124	0.118	0.094	0.117	0.147
D	0.154	0.116	0.193	0.159	0.179	0.110	0.177	0.168	0.177	0.135	0.150	0.177
E	0.187	0.145	0.212	0.144	0.164	0.131	0.101	0.154	0.140	0.099	0.158	0.103
F	0.207	0.170	0.306	0.195	0.220	0.146	0.114	0.183	0.195	0.076	0.071	0.143
G	0.258	0.157	0.340	0.158	0.166	0.078	0.146	0.140	0.121	0.139	0.137	0.000
H	0.184	0.176	0.218	0.118	0.207	0.118	0.179	0.145	0.199	0.130	0.137	
F	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	96 sup SA NP	96 sup SA NP
G	CLK 5.0 #1 1:5	CLK 5.0 #2 1:5	CLK 5.0 #3 1:5	CL39 5.0 #1 1:5	CL39 5.0 #2 1:5	CL39 5.0 #3 1:5	CL39 5.0 #4 1:5	CL39 5.0 #1 1:5	CL39 5.0 #2 1:5	CL39 5.0 #3 1:5	CL39 5.0 #4 1:5	CL39 5.0 #1 1:5
H	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1/2	1/2	1/2	1/2	CL39 5.0 #1 1:5

			mean A405	mouse Serum	
CLK	1:5	unrad	1.0 Gy	3.0 Gy	5.0 Gy
	1:10	0.171	0.173	0.214	0.278
		0.175	0.240	0.245	0.173
CL39	1:5	0.272	0.123	0.162	0.172
	1:10	0.203	0.173	0.192	0.205

do Circulate
 STNFR being
 bound by
 circulating
 CL39

mean A405 L929 Clone 39 Primary tumor cell lines from C3H mice:

1.0 Gy - #5 = 0.801	5.0 Gy #1 = 0.815
3.0 Gy - #1 = 0.701	5.0 Gy #2 = 0.760
3.0 Gy - #2 = 0.620	5.0 Gy #3 = 0.650
3.0 Gy - #5 = 0.717	5.0 Gy #4 = 0.701

7/9/98

9/4/98

9/4 - ID3 B1 Antitumor Infusion + Challenge w/ Clone 39

These experiments are designed to challenge the efficacy of ID3 B1 (an α -STNFR1 Ab) in protecting mice from Clone 39-derived B29 tumors. Mice will be injected with ID3 B1 or control Ab on day 0 (the time of tumor challenge) and again on days 3 and 6. Tumor development will be monitored daily.

9/4 (day 0) ~ 11:00 AM Inject ID3 B1 or 1B7.11 (α -TNP Ab) IP - 0.5 mg Ab in 1 ml PBS mouse using a 26 gauge needle (aliquotted using a pipetman, then inject all)

~ 2:30 PM Inject Clone 39 Subcutaneously (mid-back) 10⁶ cells 10.5 μ l of 10⁶ cells in 0.5 ml PBS using a 26 gauge needle (aliquotted using a pipetman, then inject all)

9/7 (day 3) Check mice for tumors - none at this time. Inject mice with the appropriate antitumor IP 0.5 ml containing 0.2 mg Ab in PBS

9/9 (day 5) Check for tumors:

ID3 B1 injected mice = 4 tumors
1B7.11 " " = 8 tumors

9/10 (day 6) Check for tumors:

ID3 B1 = 8
1B7.11 = 8

Inject mice with the appropriate Ab IP 0.5 ml containing 0.2 mg Ab in PBS

* note: the 1B7.11 mice actually got a total of 80 μ g of Ab - vs - 90 μ g of ID3 B1 Ab mice.

9/10 (day 7) ID3 B1 = 8
1B7.11 = 8

9/12 (day 8) Same as above. Resulted 2 tumors from 1B7.11 group + 1 tumor ID3 B1 group in

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all tumors are regressing.

to optimize dosage, route of administration,
frequency of administration, etc.

end of Ph.D. work - all Book #5 for Post-DOC
Research

3/24/98

3/24 2-3TNFRI ELISA of Purified ID3 and Clones of Limiting Dilution

- * Test fractions of ID3 purified from protein G column
- * Test 1st set of clones from limiting dilution

Procedure

- Coat w/ 2 μ g/ml goat α hTNFRI
- Block
- Add AB Supt (14937 + anti-mouse hTNFRI)
- Add appropriate dupt of test sample
- Add 2 μ g/ml goat α mouse IgG, IgA, IgM - AP conjugate
- Develop as per usual
- PNPP incubated for ~5 hours at RT (the substrate was the old stuff + some of new stuff - reaction was very pretty)

	1	2	3	4	5	6	7	8	9	10	11
A	SP20 Supt Nat	Wash #1 Nat	Wash #2 Nat	Wash #3 Nat	Wash #4 Nat	Wash #5 Nat	SAL #2 2 μ g/ml	SAL #3 2 μ g	SAL #4 2 μ g	SAL #5 2 μ g	SAL #6 2 μ g
B	1/2	1/2	1/2	1/2	1/2	1/2	4 μ g	4 μ g	4 μ g	4 μ g/ml	4 μ g
C	1/4	1/4	1/4	1/4	1/4	1/4	SP20 Supt + Binding Nat	SP20 Supt + Binding Nat	SP20 Supt + Binding Nat	SP20 Supt + Binding Nat	SP20 Supt + Binding Nat
D	4F2 7F	4F2 7F	4F2 7F	4F2 10C	4F2 B1	4F2 A12	147 F2 (ON 18)	3B11 A2	3B11 B12	3B11 B8	3B11 F7
E	2B8 B12	1A8 B9	1A8 C2	1A8 D4	1A8 D5	1A8 E9	1A8 F10	1A8 F5	1A8 G8	147 D2	4F2 D0

	1	2	3	4	5	6	7	8	9	10	11
A	0.363	0.447	0.081	0.060	0.038	0.044	1.404	1.600	1.571	1.657	1.657
B	0.897	1.260	0.078	0.039	0.032	0.039	1.456	1.316	1.412	1.472	1.572
C	0.917	1.157	0.106	0.066	0.053	0.030			0.065	0.000	
D	0.426	0.420	0.068	0.133	0.065	0.060	0.041	0.041	0.041	0.037	0.032
E	0.085	0.075	0.778	0.304	0.402	0.493	1.520	0.246	0.256	0.146	

Patched 4F2-7F, 4F, 10C and 1A8 - C2, D4, D5, E9, F10, F5, G8
to 4F2 and 147 - D2

notes: purified Ab is biologically active (in terms of binding to native TNF). Concentration may be much lower than predicted.

3B11 may be a total bust - no positive clones
and for binding to 9B in previous assay (pg. 29)